

Recombinant hepatitis B surface antigen as a carrier of human immunodeficiency virus epitopes

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SUMMARY

Eukaryotic cells transformed with a plasmid expression vector are able to synthesize and assemble HBsAg, a complex multimeric lipoprotein particle. Hybrid particles carrying HIV1 antigenic determinants were constructed and injected into monkeys. A complete immune response including neutralizing antibodies, proliferative and cytotoxic T-cell activities was obtained. Thus, such HIV/HBsAg hybrid particles could be a new approach to multivalent vaccination.

Key-words: Vaccine, Hepatitis B, AIDS, HBsAg, HIV; Presentation, Epitope carrier.

Introduction

Strategies to develop a vaccine against viral disease utilize two types of antigen: soluble antigen or antigen carried by a live vector (recombinant or attenuated virus). The potential immune response induced by each type of antigen could be different, since a live vector mainly stimulates a cellular cytotoxic response with minor or no antibody production. On the other hand, soluble antigen induces high-titred antibodies able to clear free virus and also cellular response, such as production of helper T cells and major histocompatibility complex class-II-dependent (Orentas *et al.*, 1990) or class-I-dependent (Deres *et al.*, 1989) cytotoxicity. Because we think that a live recombinant vaccine may present risks, especially in an immunodepressed population, we chose to present

antigenic determinants on a soluble antigen, the hepatitis B surface antigen (HBsAg), as a carrier. Combination of foreign peptides with HBsAg offers the opportunity to develop multivalent immunogens. Since there is an overlap between populations at risk from HBV and HIV infections, we decided to express HIV-envelope determinants as fusion proteins with the HBV-envelope protein.

As we have reported previously, eukaryotic cells transformed with a plasmid expression vector are able to synthesize and assemble a complex multimeric lipoprotein particle, the HBsAg particle (Michel *et al.*, 1984). This particle is structurally similar to the empty viral envelope normally present in the serum of infected patients and is highly immunogenic upon injection into animals and humans. The surface proteins of HBV are the translational products of a large

open reading frame, which is divided into three domains, each of which begins with an in-frame ATG codon that could be used as a translational initiation site. These domains are referred to as preS1, preS2, and S in their 5' to 3' order in the open reading frame, defining three polypeptides referred to as major (S only), middle (preS2 plus S) and large proteins (preS1 plus preS2 plus S). To ensure a correct presentation of the HIV antigenic determinants, they have been inserted into the preS2 region of the middle protein. This region, clearly not involved in particle assembly and secretion, contains an immunodominant epitope and is a well-exposed surface region.

The protective immune response to enveloped viruses usually involves the production of neutralizing antibodies against surface antigens. The envelope glycoprotein gp160 of HIV1 is a logical candidate as a subunit vaccine for this virus. To deal with antigenic competition between epitopes present within the full length of gp160, our strategy in the design of a vaccine that will confer protection against HIV is the presentation of selected epitopes to the immune system.

Cloning and expression of fusion proteins

We have developed a general system for cloning and expression of HIV fragments as fusion proteins in animal cells. In this system, any DNA fragment cloned in frame with the β -galactosidase gene in an M13-derived bacteriophage can be easily transferred in frame with the S gene to different HBsAg eukaryotic expression vectors (fig. 1). Alternatively, synthetic oligomers coding for known epitopes can be directly cloned into the eukaryotic vectors. After transfection of animal cells, the supernatants of cell cultures expressing HBsAg may be screened for the presence of the fusion protein using either HIV-specific RIA or Western blot analysis.

Structure of HIV/HBsAg particles

Using this cloning strategy, we have engineered several HIV/HBsAg hybrid particles form the HIV1_{LAI} isolate. We became especially interested in two of them, one (region 8) carrying an 84-amino acid domain (AA 384-467) involved in the binding of glycoprotein gp160 to the CD4 molecule (McDougal *et al.*, 1986) and the other, the V3 region, a 24-amino acids domain (AA 308-331) containing the principal neutralizing determinant (Goudsmit *et al.*, 1987) but also epitopes for cellular cytotoxicity, helper T cells and antibody-dependent cytotoxicity (Takahashi *et al.*, 1990).

Each domain was inserted into the pSV2S expression vector, and following transfection in animal cells, hybrid proteins were synthesized, glycosylated and assembled together with the HBsAg major protein, into 22-nm particles. These particles were secreted and detected in cell culture supernatants by an HBsAg-specific commercial ELISA. The particles were purified on a caesium chloride gradient according to their densities and were analysed by Western blot. The hybrid protein, in its different glycosylated forms, could be revealed by using anti-HIV1- as well as anti-HBV-specific monoclonal antibodies (mAb) as shown in figure 2. The presence of the HIV1 V3 domain on the HBsAg particles was confirmed by using different mAb in a sandwich RIA (Schlienger *et al.*, 1992). Because these mAb are able to neutralize HIV1 infectivity *in vitro*, this shows that the V3 domain is exposed on the hybrid particles as well as on the native HIV1 virus.

Humoral immune response to HIV/HBsAg particles

Immunization of rabbits or macaques with the purified hybrid particles resulted in an anti-

HBsAg = hepatitis B surface antigen.
HBV = hepatitis B virus.
HIV1 = human immunodeficiency virus type 1.

mAb = monoclonal antibody.
PBMC = peripheral blood mononuclear cells.
RIA = radioimmunoassay.

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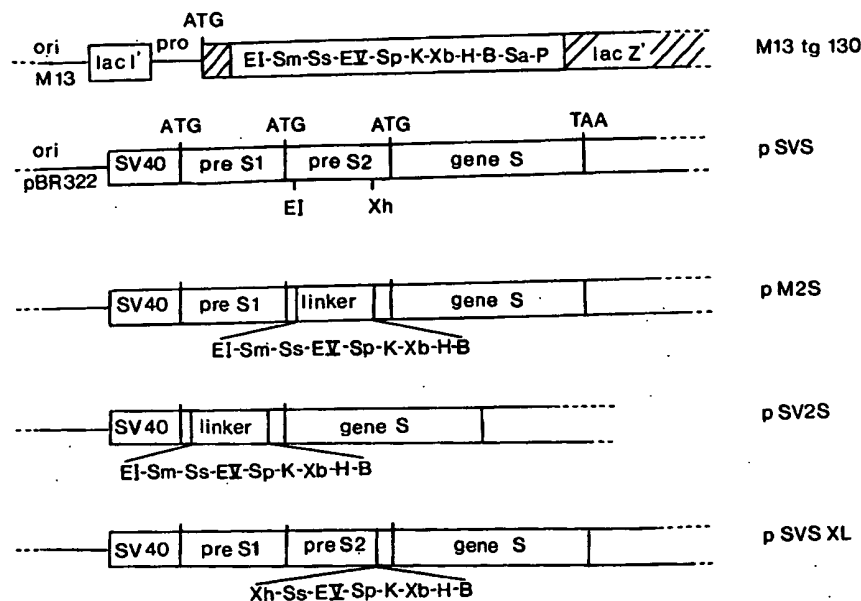


Fig. 1. Cloning and expression vectors of the HIV-envelope gene fragments. M13tg130 is the cloning vector for HIV DNA fragments.

Hatched region: N-terminal section of the β -galactosidase gene. In this region, a linker with multiple cloning sites is inserted (EI = *EcoRI*; Sm = *SmaI*; Ss = *SstI*; EV = *EcoRV*; Sp = *SphI*; K = *KpnI*; Xb = *XbaI*; H = *HindIII*; B = *BamHI*; Sa = *SalI*; P = *PstI*; Xh = *XhoI*). pSVS, pM2S, pSVS XL and pSV2S are HBsAg expression vectors. SV40 = simian virus 40.

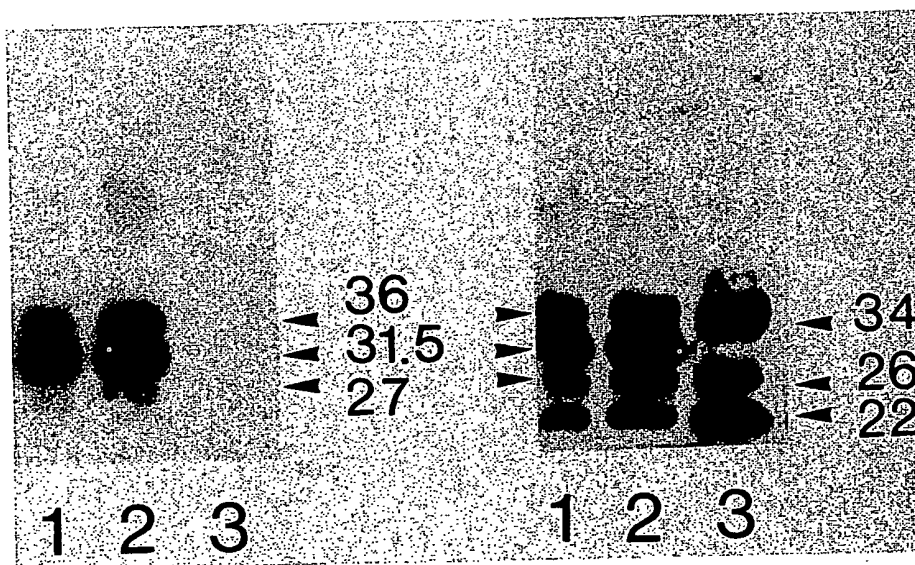


Fig. 2. Western blot analysis of an HBsAg recombinant particle.

V3/HBsAg hybrid recombinant particle 20 ng (lane 1) and 40 ng (lane 2), preS2/HBsAg native recombinant particle 40 ng (lane 3). Proteins were revealed with an anti-V3 HIV_{LAI}-specific mAb (left) or an anti-HBs-specific mAb (right). The 22- and 26-kDa bands correspond to the HBV major envelope protein, and the 34-kDa band corresponds to the HBV middle protein; the 36-, 31.5- and 27-kDa bands correspond to the hybrid middle protein.

body response directed against both parts of the hybrid particle, *i.e.* HIV and HBV (Michel *et al.*, 1988; Michel *et al.*, 1990; Schlienger *et al.*, 1992). Antibodies induced by particles carrying region 8 recognized homologous HIV1 peptides, and the recognition of gp160 glycoprotein appeared late in the course of immunization. Conversely, antibodies to particles carrying the V3 domain reacted more efficiently with the complete envelope protein than with the homologous synthetic peptide. This suggests that the domains are presented differently in the gp160 molecule: the V3 domain, readily accessible and the CD4-binding domain, buried in the protein. In both experiments, anti-HBsAg antibodies reached titres equivalent to those obtained following immunization with native particles (control animals). Thus, presentation of foreign determinants at the surface of the particles does not prevent recognition of HBsAg.

The ability of sera from immunized macaques to neutralize HIV1 infectivity *in vitro* was monitored by inhibition of HIV1-specific reverse transcriptase activity and by inhibition of syncytia formation. Rhesus monkey sera obtained after 3 injections of the V3/HBsAg hybrid particle (2 injections one month apart followed by a booster 6 months later) were able to block both free virus infectivity and a virus-induced cytopathogenic effect at the same time (Schlienger *et al.*, 1992).

Cellular immune response to hybrid particles

The ability to induce HIV-specific helper T cells is likely to be an essential requirement of any vaccine designed to prevent or control HIV infection. Helper T cells are required for both antibody and cytotoxic responses. The proliferative responses of peripheral blood mononuclear cells (PBMC) isolated from macaques before and after immunization were tested against HBsAg and HIV1 antigens. An *in vitro* specific proliferative response to HIV1 killed virus of V3/HBsAg-immunized macaques was observed after the first immunization and was maintained for several months after the second immunization. Because the V3 domain contains a helper

T-cell epitope, it is likely that the V3 domain exposed on HBsAg particles is processed in a manner similar to that which occurs when this domain is presented by the virus.

Similarly the T-cell proliferative response of lymphocytes isolated from macaques vaccinated with hybrid particles carrying region 8 were equally well stimulated with HIV1 and gp160. Moreover, a significant response was observed when purified HIV2 was used as a recall antigen, indicating cross-reactivity at the T-cell level in the recognition of the CD4-binding domain, a well conserved domain in both viruses. A specific proliferative response to HBsAg was elicited in the control animals as well as in the animals immunized with the hybrid particles. This response appeared following the first immunization and was enhanced after the booster. Thus, the processing of the hybrid particles did not alter the processing and the presentation of the HBsAg-specific T-cell epitopes.

Another major goal of vaccination is to generate immune cells able to recognize and kill infected cells. We thus examined this possibility by testing the lytic activity of fresh PBMC from immunized animals towards autologous B lymphoblastoid target cell lines. In a standard chromium release assay, PBMC from V3/HBsAg-immunized macaques were able to specifically lyse target cells infected with recombinant vaccinia viruses and expressing gp160 at the cell surface. In the same assay, PBMC from the control macaque did not lyse any target. These experiments provide evidence that HBsAg carrying an HIV1 domain is able to induce immunity to and memory of both HBV and HIV at the T-cell level.

In summary, HBsAg is a good carrier for presentation of HIV determinants, since it enabled us to obtain a large spectrum of immune response including neutralizing antibodies, T-cell proliferative response and cytolytic activities in immunized primates. This system does not exclude the possibility of combining various epitopes or different sequences of the same epitope either on the same particle or in a cocktail of particles. Since HBsAg has already proved its safety and

efficacy in human vaccination, the use of such hybrid particles could be a new approach in multivalent vaccination.

References

- Deres, K., Schild, H., Wiesmüller, K.-H., Jung, G. & Ramensee, H.-G. (1989), *In vivo* priming of virus-specific cytotoxic T lymphocytes with synthetic lipopeptide vaccine. *Nature (Lond.)*, 342, 561-564.
- Goudsmit, J., Debouck, M.C., Melen, R.H., Smit, L., Bakker, M., Asher, D.M., Wolff, A.V., Gibbs, C.J. & Gajdusek, D.C. (1987), Human immunodeficiency virus type 1 neutralization epitope with conserved architecture elicits early type-specific antibodies in experimentally infected chimpanzees. *Proc. nat. Acad. Sci. (Wash.)*, 85, 4478-4482.
- McDougal, J.S., Nicholson, J.K.A., Cross, G.D., Cort, S.P., Kennedy, M.S. & Mawle, A.C. (1986), Binding of the human retrovirus HTLV-III/LAV/ARV/HIV to the CD4 (T4) molecule: conformation dependence, epitope mapping, antibody inhibition, and potential for idiotypic mimicry. *J. Immunol.*, 137, 2937-2944.
- Michel, M.L., Pontisso, P., Sobczak, E., Malpiece, Y., Streek, R. & Tiollais, P. (1984), Synthesis in animal cells of hepatitis B surface antigen particles carrying a receptor for polymerized human serumalbumin. *Proc. nat. Acad. Sci. (Wash.)*, 81, 7708-7712.
- Michel, M.-L., Mancini, M., Sobczak, E., Favier, V., Guetard, D. & Tiollais, P. (1988), Induction of anti-HIV neutralizing antibodies in rabbits immunized with recombinant HIV-HBs antigen particles. *Proc. nat. Acad. Sci. (Wash.)*, 85, 7957-7961.
- Michel, M.-L., Mancini, M., Riviere, Y., Dormont, D. & Tiollais, P. (1990), T- and B-lymphocyte responses to HIV type 1 in macaques immunized with hybrid HIV/hepatitis B surface antigen particles. *J. Virol.*, 64, 2452-2455.
- Orentas, R.J., Hildreth, J.E.K., Obah, E., Polydefkis, M., Smith, G.E., Clements, M.L. & Siliciano, R.F. (1990), Induction of CD4⁺ human cytolytic T cells specific for HIV-infected cells by a gp160 subunit vaccine. *Science*, 248, 1234-1237.
- Schlienger, K., Mancini, M., Riviere, Y., Dormont, D., Tiollais, P. & Michel, M.-L. (1992), Human immunodeficiency virus type 1 major neutralizing determinant exposed on hepatitis B surface antigen particles is highly immunogenic in primates. *J. Virol.*, 66, 2570-2576.
- Takahashi, H., Takeshita, T., Morein, B., Putney, S., Germain, R.N. & Berzofsky, J.A. (1990), Induction of CD8⁺ cytotoxic T cells by immunization with purified HIV-1 envelope protein in ISCOMs. *Nature (Lond.)*, 344, 873-875.